

Production of Biodiesel Using Immobilized *Pseudomonas* Fluorescence

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ABSTRACT

Transesterification of vegetable oils is an important reaction that produces fatty acid alkyl esters, methyl and ethyl esters which are excellent substitutes for diesel fuel. Biodiesel prepared by catalyzed mild transesterification has become of much current interest for alternative fuel production. In the present study the ability of a commercial immobilized *Pseudomonas fluorescens* MTCC 103 to catalyze the transesterification of *Jatropha* oil and methanol was investigated. The cell of *P. fluorescens* was easily immobilized within the sodium alginate during batch process. The important parameters like reaction temperature, pH, oil/methanol molar ratio, amount of beads and reaction time was studied. From the study it was found that highest yield of biodiesel was obtained at the optimum conditions of at temperature 40°C, pH of 7.0, molar ratio of 1:4, amount of beads of 3 g and reaction time of 48 h. The physical properties of the products were analyzed and the results were compared with conventional petroleum based diesel and it was found that the product can be used as an effective alternate fuel in existing diesel engine without any hardware engine modifications.

Keywords – Biodiesel, conventional, environmental, economical, Transesterification.

1. INTRODUCTION

Increasing energy demand, climate change and carbon dioxide (CO₂) emission from fossil fuels make it a high priority to search for low-carbon energy resources. Bio fuels represent a key target for the future energy market that can play an important role in maintaining energy security. It is primarily considered as potentially cheap, low-carbon energy source. Most life-cycle studies of bio fuels have found that bio-ethanol made from corn or sugarcane generally reduces greenhouse gases, replacing gasoline (petrol) [1]. Biodiesel can be produced from any biological feedstock that contains oil or animal fat, through a chemical process (called “transesterification”), reacting feedstock oil or fat with methanol and a potassium hydroxide catalyst. Soybean, sunflower, rapeseed and palm fruit are oil-seed

crops used to produce biodiesel. Animal fat, or even used frying oil can be used to produce biodiesel too. Biodiesel also includes synthetic diesel fuel made through gasification or some other approach. The biodiesel process involves well-established technologies that are not likely to change significantly in the future, but efficiency can be improved by large scale production. Biodiesel, which is the name of a clean burning, renewable diesel fuel replacement made from vegetable oils or animal fats. The methyl ester of vegetable oil or what we now call Biodiesel is very similar to the petrochemical based diesel fuel that it replaces so does not require any vehicle or storage modifications.

Renewable source of energy undergo quick recycling, reproducing and replacement within a reasonable time maintain themselves (like biodiesel, LPG, biogas etc.) but nonrenewable energy source which do not undergo recycling and replacement for example fossil fuels (like coal, petroleum etc.) The main advantage of using this alternative fuel are its renewability, better quality exhaust gas emission, its biodegradability and, given that all the organic carbon present is photosynthetic in origin, it does not contribute to a net rise in the level of carbon dioxide in the atmosphere, and consequently to the greenhouse effect (if the carbon dioxide emitted from fertilizer production is not taken into account).

Biodiesel can be used as a pure fuel or blended with petroleum in any percentage, B5, a blend of 5% Biodiesel and 95% by volume of petroleum diesel has shown improvements in performance, enhanced lubricity and some reduction in emissions. A blend of 20 percent by volume Biodiesel with 80 percent by volume petroleum diesel called B20, has demonstrated significant environmental benefits with a minimum increase in cost. The other way to fuel a diesel engine with vegetable oil is to reduce the oil's viscosity before it gets into the tank and in this way; neither the engine nor vehicles need modification.

1.1 BIODIESEL

Biodiesel is defined as: a fuel composed of mono-alkyl esters of long chain Fatty acids derived from vegetable oils or animal fats, designated B100. A “mono-alkyl ester” is the

product of the reaction of a straight chain alcohol, such as methanol or ethanol, with a fat or oil (triglyceride) to form glycerol (glycerin) and the esters of long chain fatty acids [2]. Biodiesel, defined as “a substitute for, or an additive to diesel fuel that is derived from the oils and fats of plants and animals”. The transesterification reaction of oil to give mixtures of fatty acids methyl esters. Biodiesel almost completely eliminates lifecycle carbon dioxide emissions. When compared to petro-diesel it reduces emission of particulate matter by 40%, unburned hydrocarbon by 68%, carbon monoxide by 44%, sulphates by 100%, poly cyclic hydrocarbon (PAH) by 80%, and the carcinogenic nitrate PAH by 90% on an average. The use of biodiesel complements the working of the catalyst and can help a current EURO-1 motor attain the EURO-111 standards [3].

1.2 PETRODISEL

Diesel is produced from petroleum, and is sometimes called petro diesel when there is a need to distinguish it from diesel obtained from other sources. “As a hydrocarbon mixture, it is obtained in the fractional distillation of crude oil between 250°C and 350°C at atmospheric pressure”. Petro diesel is considered to be a fuel oil and is about 18% denser than gasoline. Diesel fuel, however, often contains higher quantities of sulfur; reduction in level of sulfur is essentially required in diesel fuels. High levels of sulfur in diesel are harmful for the environment. It prevents the use of catalytic diesel particulate filters to control diesel particulate emissions, as well as more advanced technologies, such as nitrogen oxide (NOx) adsorbers (still under development), to reduce emissions. However, lowering sulfur also reduces the lubricity of the fuel, meaning that additives must be put into the fuel to help lubricate engines. Biodiesel is an effective lubricity additive.

1.3 CHEMICAL COMPOSITION OF BIODIESEL

Most of biodiesel consists of alkyl usually methyl esters instead of the alkanes and aromatic hydrocarbons of petroleum diesel. However biodiesel has combustion properties very similar to regular diesel including combustion energy and cetane ratings paraffin biodiesel also exists. Due to the purity of the source, it has a higher quality than petro diesel. Petroleum derived diesel is composed of about 75% saturated hydrocarbons (primarily paraffin's including *n*, *iso*, and cycloparaffins), and 25% aromatic hydrocarbons (including naphthalene's and alkyl benzenes). The average chemical formula for common diesel fuel is $C_{12}H_{26}$, ranging from approx. $C_{10}H_{22}$ to $C_{15}H_{32}$.

Biodiesel is derived from vegetable oils. The major components of vegetable oils are triglycerides. Triglycerides are esters of glycerol (see above; an alcohol with a hydroxyl group on each of its three carbon atoms) with long-chain acids, commonly called fatty acids. The comparison of the rational names of the fatty acids with their structural formula

show the position of the double bonds is defined by numbers. The number of carbon atoms is counted by beginning with the first carbon having the functional group defining the fatty compound as acid or ester.

1.4 COMPARISON BETWEEN PETRO & BIODIESEL

Biodiesel can be obtained from vegetable oil and animal fats (bio-lipids, using transesterification). Biodiesel is a non-fossil fuel alternative to petro diesel. It can also be mixed with petro diesel in any account in modern engines, though when first using it, the solvent properties of the fuel tend to clear out all the garbage that has built up from the petro diesel and can clog fuel filters.

Properties	Petrodiesel	Biodiesel
Density 20°C	0.815	0.891
Viscosity 20°C	4.736	6.483
Flash point	>60	>105
Residue carbon w/w	<0.025	<0.005
Ash % w/w	<0.025	<0.005
Heat KJ/Kg	44950	39425

Table: 1 Comparison properties of Petro diesel and Biodiesel

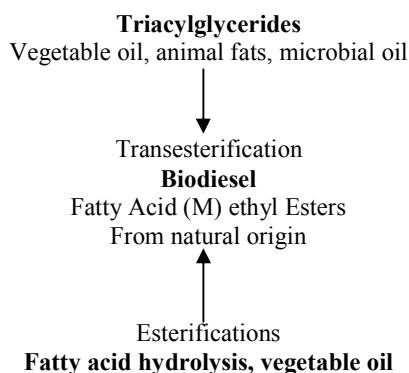
Biodiesel has a lower gel point than regular diesel, but is comparable to diesel. This can be overcome by using a biodiesel/petro diesel, blend, or by installing a small heater in your fuel system, but this is only necessary during the colder months. There have been reports that a diesel biodiesel mix results in lower emissions than either can achieve alone. A small percentage of biodiesel can be used as an additive in low-sulfur formulations of diesel to increase the lubricating ability that is lost when the sulfur is removed.

1.4 PRODUCTION PROCESS (TRANSESTERIFICATION)

Chemically, transesterification (also called alcoholysis) means taking a triglyceride molecules or a complex fatty acid, neutralizing the free fatty acids, removing the glycerin and creating an alcohol. A catalyst is usually used to improve the reaction rate and yield. The transesterification reaction is an equilibrium reaction. In this reaction, however a large amount of methanol was used to shift the reaction equilibrium to the right side and produce more methyl esters, the proposed product. Alcohols are primary or secondary monohydric aliphatic alcohols having 1-8 carbon atoms. Among the alcohols that can be used in the transesterification reaction are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used most frequently.

Ethanol is a preferred alcohol in the transesterification process compared to methanol because it is derived from agricultural products and is renewable and biologically less objectionable in the environment, however methanol is used

because of its low cost and its physical and chemical advantages (polar and shortest chain alcohol).



The transesterification reaction can be catalyzed by alkalis, acids or enzymes. Several alcoholysis catalysts, known to be effective for reactions between simple alcohols and oil, were evaluated and found to be ineffective toward alcoholysis of ethylene glycol with oil under traditional reaction conditions.

Commonly acids are used in this process; these include sulfuric acid. A high yield of alkyl esters is achieved however high temperature is required to speed up the process. Transesterification process under acid catalyses condition needs to be done in absence of water in order to avoid formation of carboxylic acid, which reduces the yield alkyl ester. The proper determination of the alcohol/vegetable oil ratio influences the final yield. With the use of this type of catalyst excess amount of alcohol is required to complete the transesterified process.

Base catalyzed transesterification process is more efficient and is less corrosive than acid catalyses process which makes it more applicable to industrial use. Base catalysts for transesterification process includes metal hydroxides like sodium or potassium hydroxide, carbonates and metal alkoxides. Metal hydroxides are readily available but are less reactive. Increasing the concentration of the catalyst in the mixture accelerates the reaction. The condition of activity of this catalyst is under anhydrous conditions. However, this cannot be avoided as water is formed during the reaction between hydroxides and alcohol. The yield of alkyl esters is reduced as water hydrolyses the ester, forming soap. Soap forms an emulsion with glycerol making recovery of glycerol difficult. The commonly catalyst used is sodium hydroxide due to its economical availability. However, potassium hydroxide has been reported to be a superior catalyst in the production of biodiesel. Fatty acid was covalently attached to lipase from *Phycomycetites*, yielding a modified lipase of higher specific activity in hydrolytic reaction in organic solvents [4]. The initial rate of transesterification by modified lipase was almost 40 times that of native lipase in organic solvent.

2. MATERIALS & METHODS

2.1 MICROORGANISM (CULTURE)

Pseudomonas fluorescens, MTCC -103 strains procured from Microbial Type Culture Collection, Chandigarh (India) was used in this work. The culture was maintained on agar slant & stored at 4°C in refrigerator. The non-edible crude *Jatropha* oil was purchased commercially and was stored at 4°C to avoid rancidity of the vegetable oil.

2.2 MEDIA & CULTIVATION

Medium for *Pseudomonas fluorescens* was used as MTCC 103 (Nutrient agar). Beef Extract 1g, Yeast Extract 2g, Peptone 5g, NaCl 5g, Distilled H₂O 1 lit, Agar 30g, pH=7. Nutrient agar medium was used for revive of culture. The culture was maintained on nutrient agar medium. After three days incubation at 25°C, the agar slants were stored at 4°C. Inocula were grown aerobically in 250 ml Erlenmeyer flask containing the liquid medium at 25°C in an Orbital Shaker at 200 rpm for 24 h. Active cells were centrifuged in a centrifuge (3000 rpm) for 5 min. after that washed with sterile water. This step repeated at least three times & then used as inoculums.

2.3 CELLS IMMOBILIZATION BY ENTRAPMENT

The sodium alginate entrapment of cells was performed according to the standard method. Alginate solution with a concentration range of 0.5 - 10% was used for the cell immobilization and was prepared by dissolving sodium alginate in boiling water and autoclaved at 121°C for 15 min. Both alginate slurry and cell suspension was mixed and stirred for 10 min to get a uniform mixture the alginate/ cell mixture which was extruded drop by drop into a cold sterile 0.2 M CaCl₂ solution through a sterile 5 ml pipette from 5 cm height and kept for curing at 4°C for 1 h. The beads were hardened by resuspending into a fresh CaCl₂ solution for 24 h at 4°C with gentle agitation. Finally these beads were washed with distilled water to remove excess calcium ions and untrapped cells. When the beads are not being used, they are preserved in 0.86% sodium chloride solutions in the refrigerator.

2.4 CALCULATIONS

2.4.1 Specific gravity:- The specific gravity was determined with a specific gravity bottle or pycnometer. The temperature at which the specific gravity was determined 30°C/30°C. The specific gravity was calculated by the formulae:

$$\text{Specific gravity at } 30^{\circ}\text{C} / 30^{\circ}\text{C} = \frac{A - B}{C - B}$$

Where *A* = weight in g of the specific gravity bottle with oil at 30°C,

B = weight in g of the specific gravity bottle, and

C = weight in g of the specific gravity bottle with water at 30°C

The specific gravity of the fuels at 15.6 °C was also employed to find the specific gravity at other temperatures by using the ASTM D1250 Petroleum Measurement Tables (1953)[13,14].

2.4.2 Viscosity of oil: Viscosity is measured by Oswald-viscometer from the following formula

$$\text{Viscosity } (\eta) = n_w \frac{t_s \times d_s}{T_w \times d_w}$$

Where

η_s = Viscosity of the sample in cp at room temperature

η_w = Viscosity of the water in cp at room temperature

t_s = time of flow of sample (vol=v) in sec.

t_w = time of flow of water (vol=v) in sec.

d_s = density of sample in g/l at room temperature

d_w = density of water in g/l at room temperature

2.4.3 Acid Value: Weigh accurately about 2 g of oil sample dissolve it in 25 ml of alcohol (ethanol) and add 2 to 3 drop of 1 % phenolphthalein titrated against by 0.1 N NaOH gives pink color (end point).

$$\text{Acid Value} = \frac{N E V}{W}$$

2.4.4 Iodine value: Iodine value was calculated by-

$$\text{Iodine Value} = \frac{V_b - V_s}{W} N 12.69$$

Where Wt. of oil = w g

Vol. of hypo used against sample solution = V_s ml

Vol. of hypo used against blank solution = V_b ml

Normality of hypo solution = N

2.4.5 Saponification value: It was also calculated by the formula

$$\text{Saponification value} = \frac{56.1(B - S) N}{W}$$

Where

B = volume in ml of standard hydrochloric acid required for the blank

S = volume in ml of standard hydrochloric acid required for the sample

N = normality of the standard hydrochloric acid, and

W = weight in g of the material taken for the test

W g of the sample requires X mg KOH

1 g of the sample requires x/w mg KOH

Specific gravity, Iodine value and others was measured using the standard method [15&16]. Capillary stopper relative specific gravity bottle (pycnometer bottle) of 50 ml capacity was used to determine specific gravity of biodiesel.

3. RESULT & DISCUSSION

In present work experiments were carried out to growth of culture, *Pseudomonas fluorescens* procured from MTCC, Chandigarh and maintained it on agar slant, cell immobilized by entrapment, characteristics of Jatropa oil, conversion of oil to biodiesel by transesterification and to determine the effects of various parameter (temperature, pH, reaction time, amounts of beads, oil alcohol molar ratio) on the production of biodiesel by transesterification reaction.

3.1 DETERMINATION OF CHARACTERISTICS OF JATROPHA OIL

Chemically oil and fats are triesters of glycerol. Depending upon the physical state of an ester it is called fat (if solid) or oil (if liquid) at room temperature. The following observations were calculated as shown in Table2.

S. N.	Characteristics of oil	Value
1.	Saponification value	13.89 mg KOH/g oil
2.	Specific gravity at 30°C / 30°C	0.92379
3.	Viscosity of oil	52.780 mm ² /s at 303 K
4.	Acid value (with ethanol)	9.8 mg KOH/g oil
	Acid value (with ethanol)	13.2 mg KOH/g oil

Table: 2 Characteristics of Jatropa Oil

3.2 OPTIMIZATION OF TRANSESTERIFICATION REACTION FOR BIODIESEL PRODUCTION

3.2.1 Effect of temperature on the transesterification reaction: Effect of temperature on the transesterification reaction was examined at the temperature range from 30°C to 45°C. Four conical flasks containing 50 ml of Jatropa oil, 3 ml hexane, methanol (1:4 molar ratio of oil/methanol) and 3g beads of immobilized cell concentration. These flasks incubated four different temperatures respectively 30°C, 35°C, 40°C and 45°C at 100 rpm for 48 h. Figure 4.5.1 Shows that the highest percentage conversion at 40°C is 76% and thereafter decreases due to denature of the enzyme.

3.2.2 Effect of pH on the transesterification reaction: Effect of pH on biodiesel production from Jatropa oil using immobilized cells of *Pseudomonas fluorescens* was studied by conducting experiments with different pH as 5.5, 6.0, 6.5, 7.0, and 7.5. The constant reaction mixtures such as 50 ml of Jatropa oil, 3 ml hexane, methanol (1:4 molar ratio of oil/methanol) and 3g immobilized cell concentration in a conical flasks. These flasks were incubated at a constant optimum temperature of 40°C for 48 h reaction period. Figure 2, Shows that the yield of biodiesel was found to be increasing with increasing pH and maximum 76% was

obtained at pH 7.0. Thereafter decreased when pH increased beyond 7.0.

3.2.3 Effect of Effect of reaction time on the transesterification reaction: Effect of time on conversion of fatty acid into their corresponding esters increases with

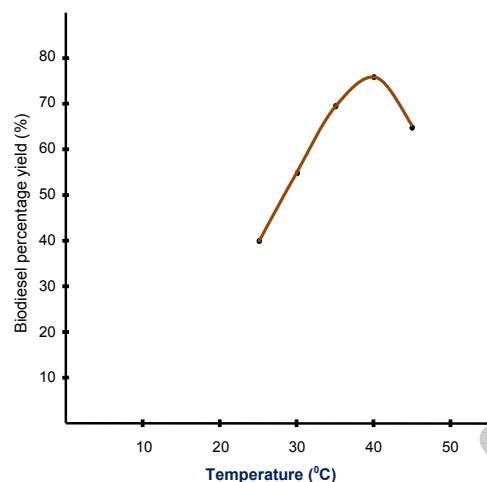


Fig. 1 Effect of temperature on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

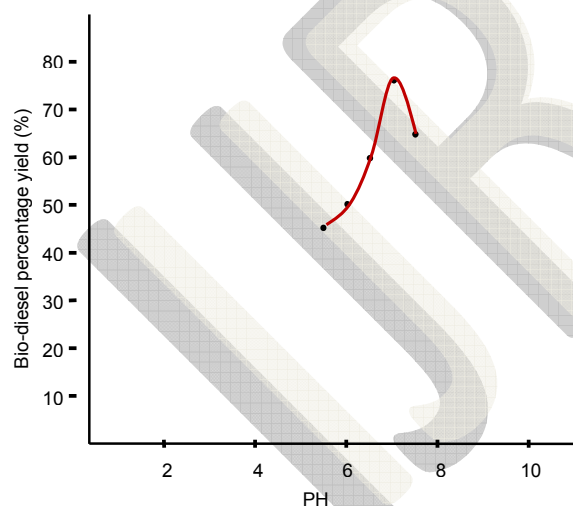


Fig 2. Effect of pH on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

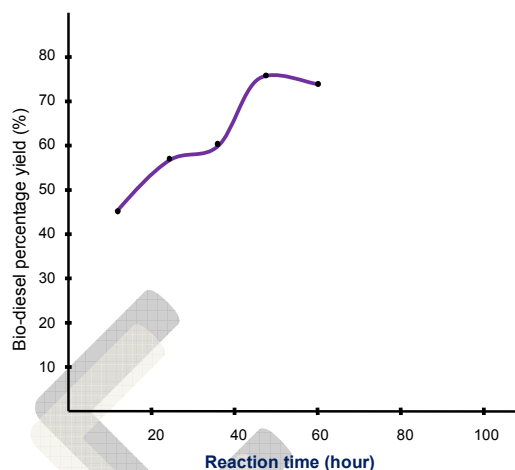


Figure 3. Effect of reaction time on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

-time. The reaction mixture containing 50 ml of oil, 3 ml hexane, methanol (1:4 molar ratio of oil/methanol) and 3g beads of immobilized cells was taken in a conical flask. These flasks were incubated at optimum temperature 40°C in a different time interval as 12, 24, 36, 48 and 60 h. Figure 3, Shows that on increasing the reaction time, the percentage yield of biodiesel increases till 48 h. thereafter slightly decreases, further increases in the reaction time does not have effect on the production of biodiesel.

3.2.4 Effect of amount of beads on the transesterification reaction: The amount of immobilized beads also effect on production of biodiesel from Jatropha oil. Experiments were carried out at different amount of beads as 1, 2, 3, 4, and 5g at constant levels of 50 ml Jatropha oil, 3 ml hexane and methanol (1:4 molar ratio of oil/methanol) at optimum condition of temperature 40°C, pH 7.0 and reaction time 48 h.

Figure 4 Shows that on increasing the amount of beads the percentage yield of biodiesel increases till 4 g (74%) and thereafter slightly decreases. Further does not have effect on production yield. So 4g bead was chosen at the optimum amount of beads.

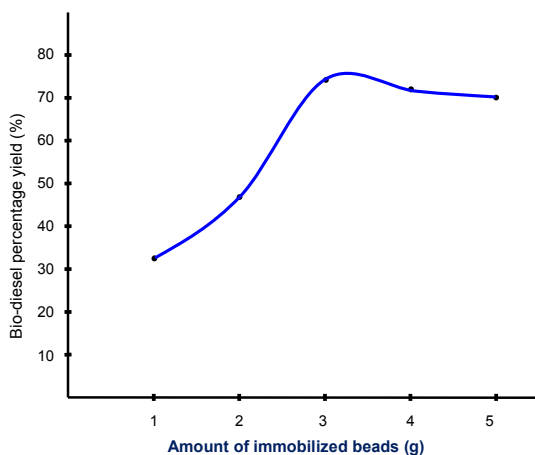


Figure 4. Effect of immobilized beads on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

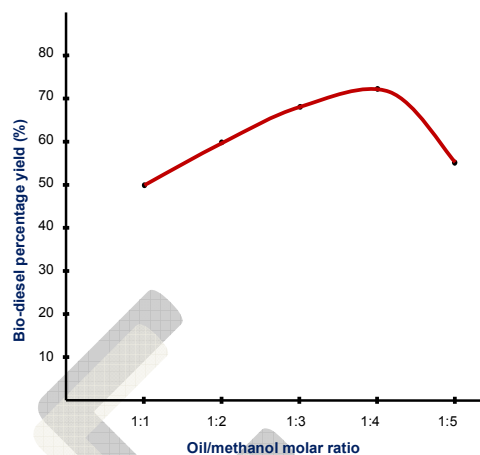


Figure 5. Effect of oil/methanol ratio on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

3.2.5 Effect of oil / alcohol molar ratio on the transesterification reaction: Oil/ alcohol molar ratios are also effect on the yield of biodiesel, because to shift the transesterification reaction in forward direction. It is necessary to use either an excess amount of alcohol or to remove one of the products from the reaction or mixture. Experiments were carried out with different molar ratios of 1:1, 1:2, 1:3, 1:4, and 1:5 (oil to methanol) at constant levels of 50 ml Jatropha oil, 3 ml hexane and 3g immobilized cell concentration in a conical flasks. This reaction mixture incubated at optimum conditions such as temperature 40°C, pH 7.0 and reaction time 48 h. Figure 5 Shows that the increasing the molar ratio, the yield of biodiesel was found to be increasing and a maximum yield of 72% were obtained at molar ratio of 1:4. Further yield of biodiesel was found to be decreasing with increasing molar ratio beyond 1:4. It is due to the inhibition of excess methanol reduces the enzyme activity.

3. CONCLUSION

There are several methods are available for producing biodiesel but transesterification of natural oil is currently the method of choice. The purpose of the process is to lower the viscosity of oil. The transesterification was carried out using Jatropha oil and short chain alcohols such as methanol and ethanol by the immobilized cells of *Pseudomonas fluorescens* MTCC 103 under specific condition.

Optimization of various transesterification parameters such as temperature, pH, reaction time, amount of beads and oil/alcohol molar ratio; which affects the biodiesel yield. The maximum yield of 76% was obtained at optimum values of temperature 40°C, pH, reaction time 48 h, amount of beads 4g and molar ratio of oil to alcohol 1:4. The biodiesel produced was analyzed for its physical properties and compared with the petroleum based diesel. The specific gravity, viscosity slightly lower than that of diesel, whereas the diesel index is much higher and the smoke point is slightly lower.

Elevated NOx emissions have been considered as one of the major problems of biodiesel and biodiesel blends as compared to petroleum diesel in diesel engines [5-9]. Earlier combustion that causes more rapid cylinder pressure rise and higher combustion temperature was believed to be one of the main causes [10-12]. This suggests that NOx emissions could be reduced by retarding the combustion timing of the fuel in diesel engines, which can be achieved by adjusting injection timing according to the concentration of biodiesel in petroleum diesel. Therefore, a means to detect the concentration of biodiesel in its diesel blends will be necessary.

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